general, presumably mammalian, mitochondrial mRNAs are not capped[2,3].

The terminal or subterminal position of the initiator codon in human mitochondrial mRNAs may eliminate the need for a scanning process, at least an extensive one. A plausible mechanism would involve attachment of the ribosome at or near the 5′ end of the mRNA with recognition of the initiator codon either directly or after a fine adjustment. The secondary structure of mitochondrial mRNAs may be such that it would exclude all internal sites containing AUG or AUA codons, exposing only the terminal or subterminal one. It is also conceivable that the special features of mammalian mitochondrial ribosomes or some initiation factor would make the ribosomes suitable for recognizing the terminal or subterminal initiator codons. Analysis of the potential secondary structures of human mitochondrial mRNAs and binding studies with mitochondrial or other ribosomes should elucidate the mechanisms operating in the initiation of translation in human mitochondria.

The 5′ ends of most mRNA coding sequences are immediately adjacent to tRNA genes

The complete absence or minimal length of the 5′ non-coding stretch in human mitochondrial mRNAs may represent the vestige of a primitive organization, or may reflect, with other traits (for example, the relatively small size of the rRNA and tRNA species), an evolutionary trend to simplicity and economy of the mitochondrial genome[24]. In any case, the degree of proximity of the initiator codon to the 5′ end of the mitochondrial mRNAs seems to be dictated primarily by the features of gene organization of this genome, in particular, by the positions of the tRNA genes. In fact, whatever the location of the initiator codon relative to the 5′ end of the mitochondrial mRNAs, the data presented here (Fig. 5) suggest that whenever a tRNA gene exists on the 5′ side of the mRNA coding sequence, the latter starts immediately after the tRNA gene. This observation confirms and refines the results of a recent detailed transcription analysis of HeLa cell mtDNA[3]. These mapping and sequencing data strongly support the idea that the tRNA sequences represent the recognition signals for a putative processing enzyme which, by precise endonucleolytic cleavages, would release the 5′ ends of the mature mRNAs[2,3]. As will be discussed elsewhere[25], recent experimental evidence indicates that the tRNA sequences may perform a similar function in the processing steps which release the 3′ ends of the human mitochondrial mRNAs. These striking features of the organization of the human mitochondrial genome underlie the crucial part that the tRNA sequences probably play in mitochondrial RNA processing in human cells[2,3].

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1. Barrell, B. G. *et al.* in *31st Mosbach Colloquium on Biological Chemistry of Organelle Formation* (eds Weiss, H. & Schald, W.) (Springer, Berlin) (in the press).
2. Attardi, G. *et al.* in *The Organization and Expression of the Mitochondrial Genome* (eds Kroon, A. M. & Saccone, C.) 103–119 (North-Holland, Amsterdam, 1980).
3. Ojala, D., Merkel, C., Gelfand, R. & Attardi, G. *Cell* **22**, 393–403 (1980).
4. Barrell, B. G., Bankier, A. T. & Drouin, J. *Nature* **282**, 189–194 (1979).
5. Hagenbüchle, O., Santer, M., Steitz, J. A. & Mans, R. J. *Cell* **13**, 551–563 (1978).
6. Kozak, M. *Cell* **15**, 1109–1123 (1978).
7. Ojala, D., Montoya, J. & Attardi, G. *Nature* **287**, 79–82 (1980).
8. Crews, S. & Attardi, G. *Cell* **19**, 775–784 (1980).
9. Amalric, F., Merkel, C., Gelfand, R. & Attardi, G. *J. molec. Biol.* **118**, 1–25 (1978).
10. Attardi, G. *et al.* in *Proc. ICN-UCLA Symp. molec. cell. Biol.* **15**, 443–469 (1979).
11. Gelfand, R. thesis, Calif. Inst. Technol. (1980).
12. Fujimoto, M., Kuninaka, A. & Yoshino, H. *Agric. biol. Chem.* **38**, 1555–1561 (1974).
13. Donis-Keller, H., Maxam, A. M. & Gilbert, W. *Nucleic Acids Res.* **4**, 2527–2538 (1977).
14. Simoncsits, A., Brownlee, G. G., Brown, R. S., Rubin, J. R. & Guilley, H. *Nature* **269**, 833–836 (1977).
15. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. *J. molec. Biol.* **143**, 161–178 (1980).
16. Chomyn, A., Hunkapiller, M. & Attardi, G. *Nucleic Acids Res.* (in the press).
17. Thalenfeld, B. & Tzagoloff, A. *J. biol. Chem.* **255**, 6173–6180 (1980).
18. Shine, J. & Dalgarno, L. *Biochem. J.* **141**, 609–615 (1974).
19. Steitz, J. A. & Jakes, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4734–4738 (1975).
20. Ptashne, M. *et al. Science* **194**, 156–161 (1976).
21. Sherman, F., Stewart, J. W. & Schweingruber, A. M. *Cell* **20**, 215–222 (1980).
22. Hefti, E., Bishop, D. H. L., Dubin, D. T. & Stollar, V. J. *Virol.* **17**, 149–159 (1976).
23. Grohmann, K., Amalric, F., Crews, S. & Attardi, G. *Nucleic Acids Res.* **5**, 637–651 (1978).
24. Attardi, G. *Trend. biochem. Sci.* (in the press).
25. Ojala, D., Montoya, J. & Attardi, G. *Nature* **290**, 470–474 (1981).
26. Anderson, S. *et al. Nature* **290**, 457–465 (1981).

tRNA punctuation model of RNA processing in human mitochondria

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A 3′-end proximal segment of most of the putative mRNAs encoded in the heavy strand of HeLa cell mtDNA has been partially sequenced and aligned with the DNA sequence. In all cases, the 3′-end nucleotide of the individual mRNA coding sequences has been found to be immediately contiguous to a tRNA gene or another mRNA coding sequence. These and previous results indicate that the heavy (H) strand sequences coding for the rRNA, poly(A)-containing RNA and tRNA species form a continuum extending over almost the entire length of this strand. We propose that the H strand is transcribed into a single polycistronic RNA molecule, which is processed later into mature species by precise endonucleolytic cleavages which occur, in most cases, immediately before and after a tRNA sequence.

RECENT transcription mapping analysis of HeLa cell mitochondrial DNA (mtDNA) has revealed that the H-strand sequences specifying the rRNA and poly(A)-containing RNA species (which are probably specific mRNAs or their precursors) are flanked on one side, and often on both sides, by a tRNA gene[1,2] (Fig. 1). In agreement with these observations, the alignment with the mtDNA sequence (refs 3–5 and 37) of a 5′-end proximal sequence of all H strand-coded putative mRNAs has shown that the sequences complementary to these mRNAs are almost always immediately adjacent, without any intervening nucleotide, to a tRNA gene or an mRNA coding sequence[6,7].

Here, we report that each of the eight mRNAs analysed is immediately contiguous, on its 3′ side, to a tRNA gene or an mRNA coding sequence. On the basis of these and previous results[2,4,6–8], we propose a model of H-strand gene expression which involves transcription of almost the entire length of this strand in the form of a single polycistronic RNA molecule and its subsequent processing directed by the tRNA sequences.

Isolation and 3′-end sequencing analysis of mitochondrial mRNAs

The putative mRNAs were isolated from the poly(A)-containing RNA fraction of micrococcal nuclease-treated mitochondria

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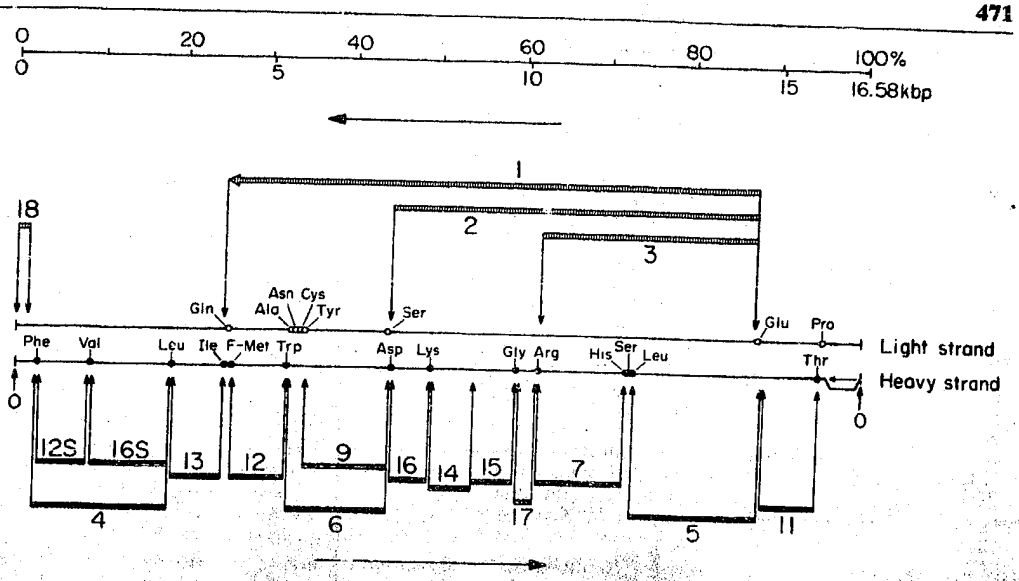

Fig. 1 Transcription map of HeLa cell mtDNA. The two mtDNA strands have been linearized at the origin of replication (0). The position and identities of the tRNA genes on the H strand (●) and on the L strand (○) were sequence (refs 16 and 37). The solid and hatched bars indicate the H-strand and L-strand transcripts, respectively; numbers refer to the various poly(A)-containing RNA species according to the classification of Amalric *et al.*[19]. The upper and lower arrows indicate the direction of L- and H-strand transcription, respectively. kbp, Kilobase pairs.

by electrophoresis through an agarose-CH_3H_7OH slab gel, elution of the individual species from the gel, treatment with RNase-free DNase and re-run of each species on a separate gel of the same type, as described elsewhere[6].

Partial sequence analysis of the mRNAs was carried out by an adaptation of the minus sequencing method using 'phased' oligo (dT) primers and reverse transcriptase[9,10], as described in Fig. 2 legend. In the transcriptase reaction, labelled primers and unlabelled dNTPs[11] were used rather than unlabelled primers and [α-^{32}P]dNTPs, as in the usual procedures; in the latter approach, the probable presence of contaminating oligonucleotides resulting from the DNase treatment (which could function as primers) was expected to complicate considerably the interpretation of the results.

To determine the correct primer to be used with the RNA being investigated (and thus the identity of the nucleotide adjacent to the poly(A) tail) each of three 5'-end-labelled primers [$p(dT)_8$-dA, $p(dT)_8$-dC and $p(dT)_8$-dG] was tested for its ability to prime cDNA synthesis using the RNA as a template. As shown previously[9,10], the presence of a specific nucleotide at the 3' end of the oligo(dT) stretch ensured that only the primer molecules hybridized with the RNA in phase (that is, those base-paired with the eight most 5'-proximal residues of the poly(A) tail and the first nucleotide on the 5' side of this tail) would promote extension synthesis by the reverse transcriptase. The band pattern formed by the products of this synthesis showed a highly variable intensity of the individual bands, and was very typical and highly reproducible for each RNA, thus providing a diagnostic test of a positive result; only limited extension synthesis was observed when the two other, non-specific primers were tested with the same RNA.

The correct oligo(dT)-dN primer identified for each RNA was incubated with the corresponding RNA, dNTPs and reverse transcriptase in four incubation mixtures, each lacking one of the four dNTPs. Figure 2 shows representative results obtained with some of the various putative mRNAs analysed. In general, in each 'minus' reaction mixture, a prominent band can be observed which corresponds to a specific ladder position. This band represents the oligo(dT) primer extended by the reverse transcriptase up to the position preceding, in the cDNA sequence, the nucleotide missing from the reaction mixture. For example, in the experiment using RNA 13, the '−A' lane shows a band at ladder position 15, the '−C' lane a band at position 25, and the '−T' lane a band at position 11. Therefore, the identities of the nucleotides at positions 16, 26 and 12 in the cDNA can be interpreted as A, C and T, respectively, and those of the corresponding nucleotides in the RNA template U, G and A. The '−G' lane shows no band above the primer, which indicates the presence of a C residue at the position in the RNA sequence corresponding to step 10 of the ladder. The other RNA species gave results similar to those obtained for RNA 13. However, in some cases, an unambiguous identification of the stop positions corresponding to all four nucleotides could not be made. In particular, RNA 12 did not produce the expected band at position 10. Recent experiments suggest that this is probably due to the melting of the short extended primer (10 nucleotides long, comprising Ts and As) from the RNA template during the chromatography step. In the case of RNA 7, the strong signal from the band of the primer (position 9) prevented the recognition of the band at position 10. In these cases, a positive identification could not be made for position 10 or 11 in the cDNA. A faint second band was observed in some cases at a position higher up than that of the first band (see the '−G' lane for RNA 12 and the '−A' lane for RNA 16). This second band was tentatively interpreted to be due to partial readthrough by the transcriptase past the first stop position (presumably because of the presence of contaminating nucleotides in the reaction mixture) and arrest at the next stop position dictated by the sequence. In some experiments, multiple prominent bands were observed in an individual lane at positions below the stop band; these generally correlated with prominent ladder bands (for example, the '−C' lane for RNA 12), and were interpreted as reflecting pauses in the transcriptase progression, as previously observed[12], and were thus disregarded.

mRNA coding sequences are immediately adjacent at their 3' end to tRNA genes or other mRNA coding sequences

The partial sequencing data determined for the 3'-end proximal segments of the putative mRNAs are shown in Fig. 3. The corresponding DNA sequences (refs 3–5 and 37) are also reported to show their alignment with the nucleotides identified in the RNAs. Our experimental data allowed unambiguous matching of the RNA and DNA sequences. The only discrepancy observed is at a residue in RNA 11 corresponding to ladder position 13, where A was found instead of G. This may reflect a sequence difference between human placenta mtDNA (used for DNA sequencing) and HeLa mtDNA; such sequence differences have been previously observed at other positions[4]. It is interesting that the base substitution mentioned above does not change the codon assignment at that position (tryptophan). It is clear from

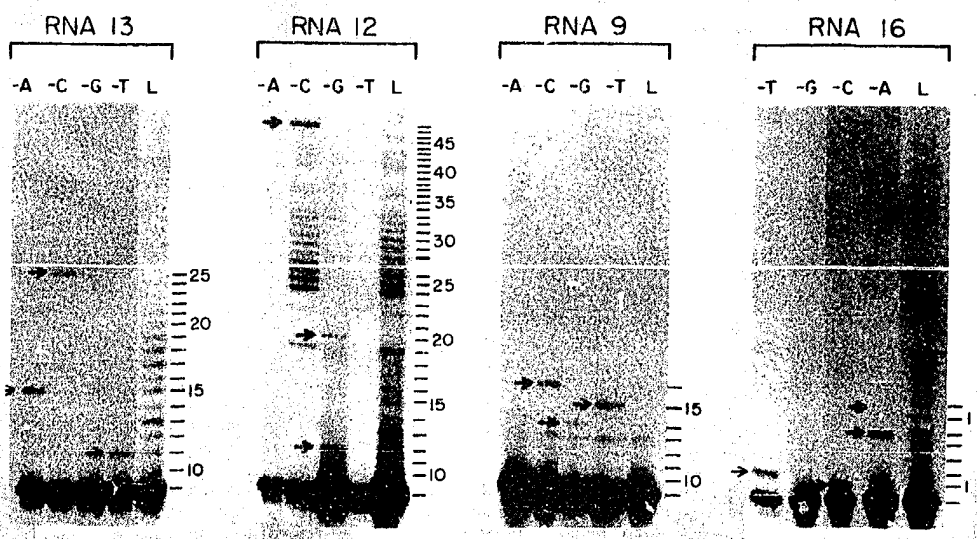

Fig. 2 Reverse transcriptase products in reactions using 'phased' oligo(dT) primers and poly(A)-containing RNAs 13, 12, 9 and 16. Partial sequence analysis of the 3'-end proximal segment of each RNA species was done by an adaptation of the 'minus' sequencing method using 'phased' oligo(dT) primers and reverse transcriptase[9,10], which will be described in detail elsewhere. Briefly, the $p(dT)_8$-dA, $p(dT)_8$-dC and $p(dT)_8$-dG primers were 5'-end labelled with [γ-^{32}P]ATP (8,000 Ci mmol^{-1}) and T4 polynucleotide kinase, after dephosphorylation with bacterial alkaline phosphatase[8], then run on a 15% polyacrylamide slab gel in Tris-borate-EDTA. After autoradiography, the band corresponding to nonanucleotides in each lane, which contained most of the radioactivity, was eluted; the final specific activity of the labelled primers was $5–10 \times 10^7$ c.p.m. μg^{-1}. To determine the nucleotide adjacent to the poly(A) tail in the RNAs to be analysed, each RNA species (0.05–0.10 pmol) was mixed at 4 °C with a 100-fold molar excess of each of the 5'-end-labelled primers and 50 mM of each of the four dNTPs in 20 μl of of 0.05 M Tris, *p*H 8.0 (25 °C), 0.05 M KCl, 0.005 M $MgCl_2$ and 0.01 M dithiothreitol. After addition of AMV reverse transcriptase (4 U), the reaction mixtures were incubated for 3 min at 39 °C. In some experiments, to facilitate the hybridization of the primer with the RNA, the mixtures were incubated for 5 min at 75 °C and then cooled for 15 min at 4 °C, before addition of the reverse transcriptase; however, no difference in results was observed. After stopping the reaction by cooling and addition of 0.01 M EDTA, the reaction products were precipitated with ethanol in the presence of 0.3 M NaCl and carrier yeast tRNA, the precipitates washed with ethanol, dissolved in 0.001 M Tris-EDTA, mixed with an equal volume of 10 M urea, heated for 2 min at 90 °C, then run on a thin (0.5 mm) 10% polyacrylamide/7 M urea sequencing gel for 3.5 h at 1,000 V. The correct oligo(dT) primer for each RNA, identified from the electrophoretic pattern of the products (see text: $p(dT)_8$-dA for RNAs 13 and 12, $p(dT)_8$-dG for RNAs 9 and 16), was incubated as described above with the corresponding RNA, dNTPs and reverse transcriptase in four reaction mixtures, each lacking one of the four dNTPs. In addition, a 'ladder' (L) reaction was performed in which all the four dNTPs were present. After stopping the reaction by quick cooling and addition of EDTA, excess primer was separated from the RNA–DNA hybrid by passage through a Sephadex G50 column equilibrated with 0.3 M NaCl, 0.01 M Tris, *p*H 7.4, in the presence of yeast tRNA carrier. The material in the void volume was ethanol-precipitated and then run on a 10% polyacrylamide/7 M urea sequencing gel. Arrows indicate the bands interpreted as corresponding to stop positions in the reverse transcriptase reaction. The numbering of the ladder steps indicates the molecular length (number of nucleotides) of the extended primers, starting from the 5'-end nucleotide.

Fig. 3 that the most 3'-proximal nucleotide identified directly in the RNA sequence either corresponds in the DNA to a residue immediately adjacent to the 5' end of a tRNA gene (RNAs 12, 16, 15, 7 and 11), or is separated from it by one to three As (RNAs 13 and 9). The most 3'-end proximal nucleotide identified in RNA 14 corresponds in the DNA to a residue separated by one A from the initiator codon (AUG) of the flanking RNA 15 coding sequence. Our experimental approach to the 3'-end sequence analysis did not allow discrimination between any 3'-terminal A encoded in the DNA and the As of the poly(A) tail. However, as in most of the mRNAs analysed the most 3'-proximal nucleotide different from A corresponds to a nucleotide in the DNA immediately contiguous to a tRNA gene, it seems reasonable to extrapolate from these results and to assume that, in the other mRNA also, where one to three As separate this nucleotide from a tRNA gene or an mRNA coding sequence, these As are similarly transcribed from the DNA.

Comparison of the 3'-end proximal sequence of the small mitochondrial rRNA from hamster cells[13] with the sequence of the corresponding segment of the human 12S rRNA gene[4] shows a high degree of homology, with the most 3'-proximal nucleotide of the hamster RNA missing in the human mtDNA sequence which just precedes the $tRNA^{Val}$ gene (Fig. 3). Therefore, it seems very likely that, as in mouse mtDNA[14], the human 12S rRNA gene extends to the $tRNA^{Val}$ gene, thus following the same rule described for the mRNA coding sequences. No direct information about the 3'-end proximal sequence of human 16S rRNA is available; however, comparison of the length of this RNA determined from S_1 protection data (~1,600 nucleotide)[15] with the length of the interval between the $tRNA^{Val}$ and the $tRNA^{Leu}$ genes (1,559 nucleotides)[4] strongly suggests that the 3' end of the 16S rRNA corresponds to a residue in DNA immediately adjacent, or very close, to the $tRNA^{Leu}$ gene.

In view of the exceedingly small amount of material available, no 3'-end sequence information was obtained for either RNA 5 or 17. However, the almost perfect correspondence between the molecular size of RNA 17, as estimated from S_1 protection data (340 nucleotides), and the length of the DNA sequence between $tRNA^{Gly}$ and $tRNA^{Arg}$ (346 nucleotides)[2], and the previously demonstrated juxtaposition of the $tRNA^{Gly}$ gene with the 5' end of the RNA 17 coding sequence[7] strongly suggest that the 3' end of the sequence specifying RNA 17 is immediately adjacent to the $tRNA^{Arg}$ gene. Similarly, the mapping contiguity of the sequences coding for RNAs 5 and 11 (ref. 2) probably reflects their immediate juxtaposition at the nucleotide level.

Most mitochondrial mRNAs terminate with U or UA

Most of the 3'-end proximal sequences of the mitochondrial mRNAs terminate with U or UA (Fig. 3). The significance of this observation has been clarified by the analysis of the human mtDNA sequence (refs 16 and 37). It has been found that many reading frames, including those corresponding to the mRNAs mentioned above, lack a stop codon, and that, in these cases, a T or TA follows the last sense codon and immediately precedes a tRNA gene or another reading frame. This observation has led to the suggestion that poly(A) addition to the 3'-terminal U or UA of the transcripts of these reading frames may create the missing stop codon. The 3'-end proximal sequences determined here for the mitochondrial mRNAs strongly support this model for polypeptide chain termination in human mitochondria. The

reading frames corresponding to RNAs 9 and 16 have stop codons AGA and UAG, respectively[3,5] (AGA is probably a stop codon in human mitochondria[17]). The observation that in these mRNAs there is a 3′ noncoding stretch extending to the 5′ end of the flanking tRNA gene indicates that, as in the case of the 5′ terminus of the human mitochondrial mRNAs[7], the rule dictating the possible presence and length of a 3′-end segment flanking the polypeptide coding sequence is the position of the tRNA genes in the DNA sequence.

The tRNA punctuation model of H-strand gene expression

The sequence data discussed above and in the accompanying paper[7] and the results of previous work[4,8] indicate that the H-strand sequences coding for the rRNAs, poly(A)-containing RNAs and tRNAs are immediately contiguous to each other, extending continuously from coordinate 2/100 to coordinate 95/100 (relative to the origin 0/100). This arrangement is consistent with a model of transcription of the H strand in the form of a single molecule which is processed by precise endonucleolytic cleavages before and after each tRNA sequence to yield the mature products or, in some cases, processing intermediates, like the putative precursor of the rRNAs (RNA 4) and the precursor of RNA 9 (ref. 2).

Recent work has indicated a concentration of H-strand nascent transcripts in the quadrant of the mtDNA *Hpa*II map which is adjacent to the origin of replication in the direction of H-strand transcription[18] (Fig. 4*a*). These results strongly suggest that the region of mtDNA around the origin of replication contains an initiation site for H-strand transcription—such a site may thus represent the promoter of the single transcript postulated here. Furthermore, the failure to detect any giant-size H-strand transcripts either associated with transcription complexes[18] or as discrete species[2,19], in contrast to the occurrence of discrete giant-size L-strand transcripts, points to the possibility that the processing of the H-strand transcripts proceeds while they still reside on the mtDNA transcription complexes. These results taken together support a model in which transcription of the H strand starts near the origin of replication and proceeds uninterruptedly at least up to the distal end of the D-loop, while the growing chains are processed to yield the mature rRNAs, poly(A)-containing RNAs and tRNAs (Fig. 4*b*).

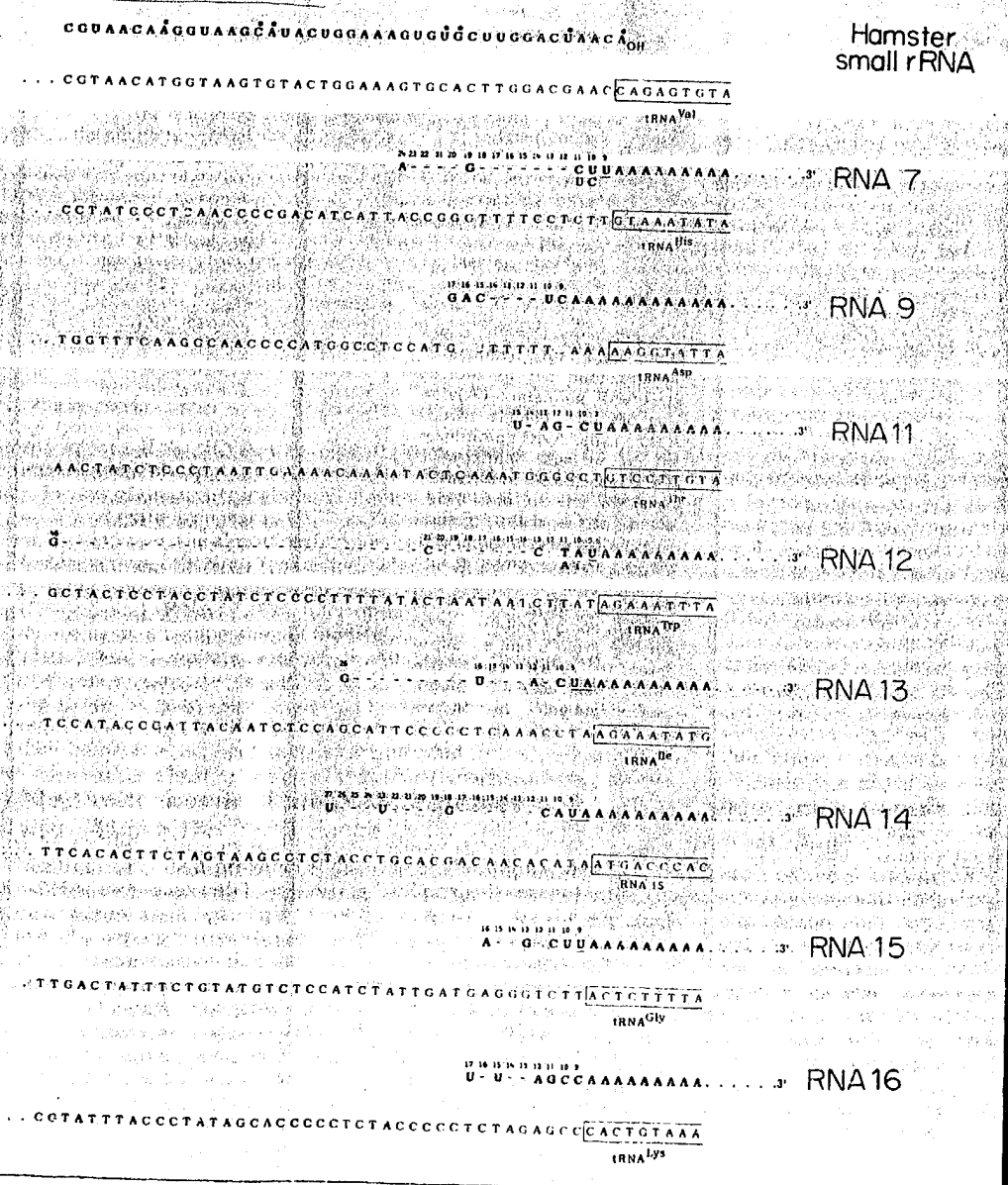

Fig. 3 Partial sequencing data determined for the 3′-end regions of the mitochondrial poly(A)-containing RNAs are shown aligned with the corresponding DNA sequences. A 5′-proximal segment of the sequence of the adjacent tRNA genes and of the RNA 15 coding stretch is also shown. The figure also includes the 3′-end proximal sequence of the hamster small mitochondrial rRNA[13], together with the corresponding human mtDNA sequence and a portion of the contiguous $tRNA^{Val}$ coding sequence. Asterisks indicate the non-homologous nucleotides.

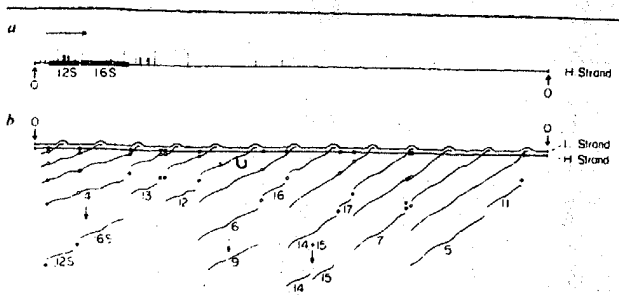

Fig. 4 Proposed model for mtDNA H-strand transcription. *a*, Distribution in the mtDNA H strand of the hybrids formed with nascent RNA chains isolated from transcription complexes (modified from ref. 18). Each tick marks the position in the *Hpa* II map (not shown) of one end of each hybrid, while the other end is arbitrarily localized at the closest, origin-proximal *Hpa* II site; long and short ticks refer to abundant and rare hybrids, respectively. *b*, Diagrammatic representation of the processing of nascent mitochondrial RNA chains in the transcription complexes: ●, tRNA gene; ○, mature tRNA.

In the processing of the primary transcripts, the secondary structure of the tRNA sequences may represent the main recognition signal, providing the punctuation in the reading of mtDNA information[1]. In fact, these sequences may acquire a clover-leaf configuration while they are still a part of the nascent transcripts, and the processing enzyme(s) may recognize this structure or a portion of it. It is interesting that the *Escherichia coli* RNase P[20-23] and a similar *in vitro* enzymatic activity from *Bombyx mori*[24] catalyse, in the tRNA precursors, precise endonucleolytic cleavages at the 5′ terminus of the mature tRNAs, while the production of the 3′ terminus of mature tRNAs in *E. coli*[22,25] and *B. mori*[24] involves a 3′→5′ exonuclease activity, following an endonucleolytic event at some distance from the 3′ end. There is good evidence to indicate that the RNase P recognizes some aspect of the structural conformation of the tRNA rather than the nucleotide sequence at its cleavage sites in the tRNA precursors[26,27]. It is possible that two enzymatic activities analogous to RNase P, but with different specificities for the 5′ and 3′ terminus of the tRNAs, are involved in the processing of the H-strand primary transcripts in human mitochondria.

There are a few processing sites in the H-strand transcripts where no tRNA sequences have been found (see Fig. 1)—at such positions, it is conceivable that the processing enzyme(s) recognizes, on one or the other side of the cleavage point, a stem-and-loop structure resembling a portion of a tRNA configuration.

Polyadenylation occurs at endonucleolytic cleavage sites of primary transcripts

One interesting observation is that all the RNA species other than tRNAs which derive from the primary processing of the nascent H-strand transcripts are polyadenylated. This suggests that polyadenylation may be linked in some way to the processing step that releases the 3′ end of these RNA products from the flanking tRNA sequences, independently of the functional role of the products. This interpretation may account for the polyadenylation of the presumptive rRNA precursor[2,28]. The small fraction of 16S rRNA which binds to oligo(dT)-cellulose[2,19] probably represents incompletely processed 16S rRNA molecules with residual poly(A) tails. It is interesting to note that, in nuclear RNA (both cellular and viral), addition of poly(A) to the 3′ ends is a rapid event which seems to precede splicing[29-31]; furthermore, at least in most of the transcripts in adenovirus 2 and SV40 infection, the acceptor sites for polyadenylation are generated by endonucleolytic cleavages of larger primary transcripts[29,32].

Implications for mitochondrial RNA metabolism

A recent estimation of the steady-state amounts of the mitochondrial mature RNA species has indicated that the molar amount of 12S rRNA is about 60 times higher than that of the most abundant mRNA, RNA 16, with moderate variations in the amounts of the different H strand-coded mRNAs[33,34]. Because the half life of the 12S rRNA is only 2–5 times longer than that of the mRNAs,[33,34] a difference in the rate of synthesis must be the main factor determining the large difference in amount between the rRNA and mRNA species. In fact, a 50–100-fold higher rate of synthesis has been estimated for 12S rRNA as compared with the mRNAs[33,34]. Such a difference in rate of synthesis between rRNA and mRNA species could suggest the existence of more than one promoter for H-strand transcription. However, in view of the regular butt-jointing of the sequences coding for all the H-strand transcripts, a more plausible interpretation is that the H strand is in fact transcribed in the form of a single molecule, but that not all transcripts are completed before being released from the template. Premature termination of the transcripts beyond the rRNA cistrons would in fact give a larger molar yield of the rRNA species relative to that of the mRNAs or most of the tRNAs[1]. Late in adenovirus 2 infection, a premature termination of transcription, which produces a four- to six-fold greater molar amount of RNA synthesized from the first 2,000 nucleotides of the transcriptional unit than from any other region, has been previously well documented[35], and recent evidence has strongly suggested premature termination in heterogeneous nuclear RNA synthesis[36]. It is attractive to think that the location of the mitochondrial rRNA cistrons close to the initiation point of H-strand transcription in animal cell mtDNA, as has emerged in the course of evolution, may have been advantageous to the cell as one which gave the rRNA cistrons a transcription advantage.

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1. Attardi, G. *et al.* in *The Organization and Expression of the Mitochondrial Genome* (eds Kroon, A. M. & Saccone, C.) 109–119 (North-Holland, Amsterdam, 1980).
2. Ojala, D., Merkel, C., Gelfand, R. & Attardi, G. *Cell* **22**, 393–403 (1980).
3. Barrell, B. G., Bankier, A. T. & Drouin, J. *Nature* **282**, 189–194 (1979).
4. Eperon, I. C., Anderson, S. & Nierlich, D. P. *Nature* **286**, 460–467 (1980).
5. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. *J. molec. Biol.* **143**, 161–178 (1980).
6. Ojala, D., Montoya, J. & Attardi, G. *Nature* **287**, 79–82 (1980).
7. Montoya, J., Ojala, D. & Attardi, G. *Nature* **290**, 465–470 (1981).
8. Crews, S. & Attardi, G. *Cell* **19**, 775–784 (1980).
9. Cheng, C. D. *et al. J. molec. Biol.* **107**, 527–547 (1976).
10. Brownlee, G. G. & Cartwright, E. M. *J. molec. Biol.* **114**, 93–117 (1977).
11. Symons, H. R. *Nucleic Acids Res.* **7**, 825–837 (1979).
12. Mills, D. R., Dobkin, C. & Kramer, F. R. *Cell* **15**, 541–550 (1978).
13. Dubin, D. T. & Baer, R. J. in *The Organization and Expression of the Mitochondrial Genome* (eds Kroon, A. M. & Saccone, D.) (North-Holland, Amsterdam, in the press).
14. Van Etten, R. A., Walberg, M. W. & Clayton, D. A. *Cell* **22**, 157–170 (1980).
15. Ojala, D. & Attardi, G. *J. molec. Biol.* **138**, 411–420 (1980).
16. Barrell, B. G. *et al.* in *31st Mosbach Colloquium biol. Chem. Organelle Formation* (eds Weiss, H. & Sebald, W.) (Springer, Berlin, in the press).
17. Barrell, B. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3164–3166 (1980).
18. Cantatore, P. & Attardi, G. *Nucleic Acids Res.* **8**, 2605–2624 (1980).
19. Amalric, F., Merkel, C., Gelfand, R. & Attardi, G. *J. molec. Biol.* **118**, 1–25 (1978).
20. Fukada, K. & Abelson, J. *J. molec. Biol.* **139**, 377–391 (1980).
21. Robertson, H., Altman, S. & Smith, J. *J. biol. Chem.* **247**, 5243–5251 (1952).
22. Schedl, P., Primakoff, P. & Roberts, J. *Brookhaven Symp. Biol.* **26**, 53–76 (1974).
23. Stark, B. C., Kole, R., Bowman, E. J. & Altman, S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3717–3721 (1978).
24. Garber, R. L. & Altman, S. *Cell* **17**, 389–397 (1979).
25. Sakano, H., Yamada, S., Ikemura, T., Shimura, Y. & Ozeki, H. *Nucleic Acids Res.* **1**, 355–371 (1979).
26. Bothwell, A. L. M., Stark, B. C. & Altman, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1912–1916 (1976).
27. Schmidt, F. J., Seidman, J. G. & Bock, R. M. *J. biol. Chem.* **251**, 2440–2445 (1976).
28. Cleaves, G. R., Jones, T. & Dubin, D. T. *Archs Biochem. Biophys.* **175**, 303–311 (1976).
29. Nevins, J. R. & Darnell, J. E. Jr *Cell* **15**, 1477–1493 (1978).
30. Schibler, U., Marcu, K. B. & Perry, R. P. *Cell* **15**, 1495–1509 (1978).
31. [illegible] C. J., Dhar, R. & Khoury, G. *Cell* **14**, 971–982 (1978).
32. F[illegible]r N. W., Nevins, J. R., Ziff, E. & Darnell, J. E. Jr *J. molec. Biol.* **129**, 643–656 (1979).
33. [illegible]eland, R. A. & Attardi, G. (submitted).
34. Attardi, G. *et al. 2nd int. Cong. Cell Biol.*, Berlin (Springer, Berlin, in the press).
35. Fraser, N. W., Sehgal, P. B. & Darnell, J. E. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2571–2575 (1979).
36. Salditt-Georgieff, M., Harpold, M., Chen-Kiang, S. & Darnell, J. E. Jr *Cell* **19**, 69–78 (1980).
37. Anderson, S. *et al. Nature* **290**, 457–464 (1981).